

Endothelial Nitric Oxide Synthase is Essential for Postpneumonectomy Compensatory Vasodilation

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Background. After pneumonectomy, the remaining lung vasculature must vasodilate to compensate for increased blood volume. We hypothesized that endothelial nitric oxide synthase (eNOS) is essential for compensatory vasodilation after pneumonectomy.

Methods. Adult, wild-type C57BL6 (WT) and eNOS knockout (eNOS^{-/-}) mice underwent left pneumonectomy and recovered under normoxic conditions. Animals were lightly anesthetized at 1, 3, 7, or 14 days after pneumonectomy, and closed chest, systolic right ventricular pressure (RVP) was recorded using fine-needle cannulation. The right ventricle to left ventricle plus septum weight ratios were measured as an index of right ventricular hypertrophy. Two additional groups of mice (WT and eNOS^{-/-}) were recovered after pneumonectomy in inhaled nitric oxide (iNO, 10 ppm), and RVP was measured on day 7.

Results. The eNOS^{-/-} mice had significantly higher preoperative RVP than did WT (17.1 ± 0.4 versus 14.2 ± 0.2 cmH₂O, $p = 0.001$). Both groups exhibited transient

periods of pulmonary hypertension after pneumonectomy. On day 1, RVP was 80% above baseline in eNOS^{-/-} mice (30.7 ± 0.8 cmH₂O) versus 42% in WT mice (20.2 ± 0.7 cmH₂O, $p = 0.0001$). The RVP returned to baseline in WT mice (16.3 ± 0.2 cmH₂O) but remained significantly elevated in eNOS^{-/-} mice (28.6 ± 0.9 cmH₂O) at day 3 and at each time thereafter ($p = 0.0001$). The iNO significantly reduced RVP in eNOS^{-/-} animals to 15.2 ± 0.3 cmH₂O ($p = 0.0001$) while having no effect in WT animals. Right ventricular hypertrophy was not observed in any group.

Conclusions. Pneumonectomy results in a transient increase in RVP. Under normal circumstances, these pressures return to baseline within 3 days. The eNOS^{-/-} mice failed to display compensatory vasodilation yet could be rescued with iNO. These results suggest that eNOS is essential for postpneumonectomy compensatory vasodilation.

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Pneumonectomy, the surgical removal of a lung, acutely reduces total lung volume, total number of alveoli, and the associated vasculature available for gas exchange. In the long term, however, pneumonectomy results in restoration of total lung volume, mass, alveolar number, and normal alveolar and capillary surface area [1]. Pneumonectomy induces a number of anatomical changes within the thorax, including increased lung distension and a mediastinal shift, that are thought to contribute to the induction of compensatory mechanisms within the remaining lung. These changes help to maintain adequate gas exchange while the remaining lung compensates for loss of lung mass. In 1996, Waller and associates [2] noted that pneumonectomy patients experienced an early period of significantly elevated pulmonary artery pressure and vascular resistance that returned to normal values within 18 hours after surgery. In

addition, postoperative noncardiogenic pulmonary edema is a serious complication of lung resection in as many as 4% of cases [3]. Increased pulmonary endothelial permeability after pneumonectomy [2] may be a predominant contributing factor to this.

Pneumonectomy directs the entire cardiac output to the remaining lung, causing vascular distention and increased parenchymal perfusion. As a consequence of these postpneumonectomy changes, there are complex cell signaling mechanisms that accommodate increased blood volume to the remaining lung. There have been numerous studies that demonstrate the beneficial and specific effects of nitric oxide on pulmonary vascular tone [4–6]. In addition, Mathisen and associates [7] have recommended immediate institution of inhaled nitric oxide as a treatment of adult respiratory distress syndrome developing after pulmonary resection [7]. The objective of the current study was to explore the intrinsic mechanisms of nitric oxide production and specifically the role of endothelial nitric oxide synthase (eNOS) in postpneumonectomy pulmonary hypertension.

We have previously shown that eNOS is critical for the compensatory growth process as eNOS gene knockout (eNOS^{-/-}) mice have severely impaired compensatory lung growth after pneumonectomy [8]. In addition, we

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have shown that eNOS protein is significantly elevated in the lung after pneumonectomy [8]. Whether alterations in pulmonary hypertension after pneumonectomy can help explain impaired compensatory lung growth in eNOS^{-/-} mice is unknown.

We hypothesized that eNOS is essential for compensatory vasodilation after pneumonectomy. We report that postpneumonectomy compensatory vasodilation is significantly impaired in eNOS^{-/-} mice and that treatment with inhaled nitric oxide (iNO) during the recovery phase attenuates pulmonary hypertension in eNOS^{-/-} animals.

Material and Methods

Adult male C57BL6, wild-type (WT) mice (n = 16) and adult male eNOS^{-/-} mice [9] on a C57BL6 background (n = 16) were subjected to left pneumonectomy through a posterior lateral thoracotomy and recovered under room air as previously described [8]. Closed chest, systolic right ventricular pressure (RVP), used as an indicator of pulmonary artery pressure, was recorded using fine-needle cannulation. The RVP was obtained in 4 animals from the WT group and 4 animals from the eNOS^{-/-} group at postoperative days 1, 3, 7, and 14. Separate groups of animals were used for each timepoint (ie, n = 4 in each group for each timepoint). The hearts of the animals were then harvested after euthanasia and right ventricle to left ventricle plus septum weight ratios were measured as an index of right ventricular hypertrophy (RVH). Two additional groups of mice (WT and eNOS^{-/-}, n = 6 in each group) were recovered in inhaled nitric oxide (iNO, 10 ppm), and RVP was measured 7 days after pneumonectomy. All animals received humane care and were used under approval from the University of Virginia's Institutional Animal Care and Use Committee.

Surgical Technique

Animals were anesthetized with intraperitoneal injection of ketamine and xylazine, and 0.5mL saline was also injected to alleviate any blood volume loss during surgery. Each mouse was shaved along the left lateral chest followed by endotracheal intubation with a 24G catheter. The surgical apparatus included a heated surface to maintain body temperature throughout surgery and recovery. The animal's left chest was opened, and the left lung was lifted into the wound. The lung hilum was tied with a ligature, and the lung was excised. The chest wall and skin were then closed in layers with sutures and surgical staples. The animals were maintained on room air using a pressure-controlled rodent ventilator (Kent Scientific, Torrington, Connecticut) during the surgery (approximately 10 minutes) and allowed to recover on room air until spontaneous respiration began. After extubation, the animals were further recovered under a heat lamp until the effects of anesthesia were minimal. When the animals were walking and demonstrating no signs of distress, they were returned to their cages and allowed to eat and drink ad libidum.

Right Ventricular Pressure Measurements

On postoperative day 1, 4 mice from the WT group and 4 mice from the eNOS^{-/-} group were randomly chosen for assessment of RVP. This process was then repeated on postoperative days 3, 7, and 14 (ie, four separate mice were used for each timepoint per group). After randomization, the mice were lightly anesthetized with ketamine and xylazine, placed in supine position, and the abdomens shaved. During spontaneous ventilation of room air, a 26G needle was passed under the xyphoid and slowly introduced into the right ventricle. Correct placement of the needle was confirmed by a RVP tracing generated using the Pulmodyn data acquisition system (Hugo Sachs Electronic, March, Germany). All animals were allowed to equilibrate during a 60-second stabilization period. After equilibration, the systolic RVP was recorded for an additional 60 seconds and used as an indicator of pulmonary artery pressure. The transducer needle was then removed, and after euthanasia, the heart was removed en bloc from each animal. Both atria were removed and discarded. The right ventricle was then carefully dissected from the intraventricular septum and weights were obtained for the right and left ventricle. The ratio of the right ventricle to left ventricle plus septum was utilized as an index of RVH.

Inhaled Nitric Oxide Recovery

A second set of WT (n = 6) and eNOS^{-/-} (n = 6) mice also underwent left pneumonectomy as described above; however, these animals were recovered in iNO chambers for 7 days. After pneumonectomy, animals were allowed to recover in room air until the effects of anesthesia were minimal (approximately 1 hour). At this point, the animals were placed in an enclosed plexiglass chamber that received a constant flow rate of 10 ppm NO blended with 21% inspired fraction of oxygen (FIO₂) and allowed to further recover with food and water ad libidum. The dose of 10 ppm was chosen to be a relatively low, physiologic dose based on previous chronic iNO studies in rodents [10]. On postoperative day 7, the animals were then individually removed from the chamber to undergo RVP measurement as described above.

Statistical Analysis

Measurements are reported as mean ± standard error of the mean (SEM). Two-way analysis of variance was used to determine whether a difference existed between study groups and postpneumonectomy day. One-way analysis of variance was used to compare the data in the second phase. Multiple comparison techniques were used to determine which groups were different for a significant *p* value in the analysis of variance. A *p* value of 0.05 or less was used to indicate a significant difference.

Results

Right Ventricular Pressure

The eNOS^{-/-} mice had subtle yet significantly higher baseline RVP than WT (17.05 ± 0.38 versus 14.25 ± 0.19

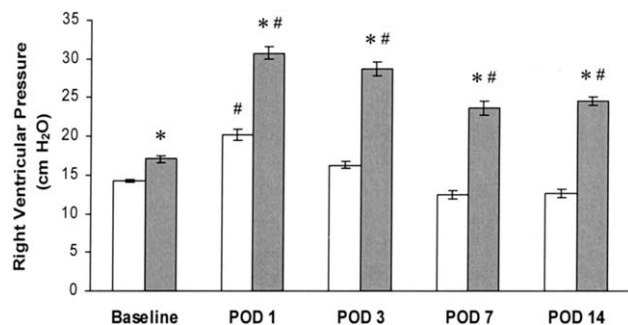


Fig 1. Right ventricular pressure (RVP) after left pneumonectomy in wild-type mice (WT [open bars]) and endothelial nitric oxide synthase knockout mice (eNOS^{-/-} [shaded bars]). The RVP was measured through a closed chest, right ventricular cannulation technique. * $p = 0.001$ versus WT for baseline, $p = 0.0001$ versus WT for days 1, 3, 7, and 14; # $p = 0.0001$ versus baseline. (Baseline = unoperated; POD = postoperative day.)

cmH₂O, $p = 0.001$). This is in agreement with prior studies that describe these mice as displaying mild but significant pulmonary hypertension [11]. Both WT and eNOS^{-/-} animals exhibited relative periods of pulmonary hypertension after pneumonectomy (Fig 1). On postpneumonectomy day 1, RVP was 80% above baseline in eNOS^{-/-} mice (30.72 ± 0.82 cmH₂O) versus 42% in WT mice (20.20 ± 0.74 cmH₂O, $p = 0.0001$). The difference in RVP was more notable on day 3, when RVP approached baseline in WT animals (16.25 ± 0.23 cmH₂O) but remained significantly elevated in eNOS^{-/-} animals (28.63 ± 0.89 cmH₂O). Significant elevations of RVP in eNOS^{-/-} mice remained consistent at every measured timepoint after pneumonectomy (days 1, 3, 7 and 14, $p = 0.0001$; Fig 1). After 14 days of recovery, the eNOS^{-/-} animals were healthy and did not display any visible signs of distress or increased mortality compared with WT animals. Post-pneumonectomy RVP in the eNOS^{-/-} group remained persistently elevated and never approached baseline or WT pressures. A two-way analysis of variance with interaction for group (eNOS^{-/-} and WT) and day (baseline, day 1, day 3, day 7, and day 14) produced p values equal to 0.0000001 for each factor (group and day) as well as for the interaction.

Inhaled Nitric Oxide Recovery

The second phase of this study was designed to test whether the persistent hypertensive effect observed after pneumonectomy in eNOS^{-/-} mice was secondary to a NO deficit. After 7 days of recovery with 10 ppm iNO, RVP was significantly reduced in eNOS^{-/-} mice (15.21 ± 0.31 cmH₂O) compared with day 7 eNOS^{-/-} mice recovered in room air (22.34 ± 0.61 cmH₂O, $p = 0.0001$; Fig 2). This reduced level was similar to the RVP observed in baseline eNOS^{-/-} mice (17.14 ± 0.60 cmH₂O; see Fig 1). The RVP in WT mice was not significantly altered by iNO.

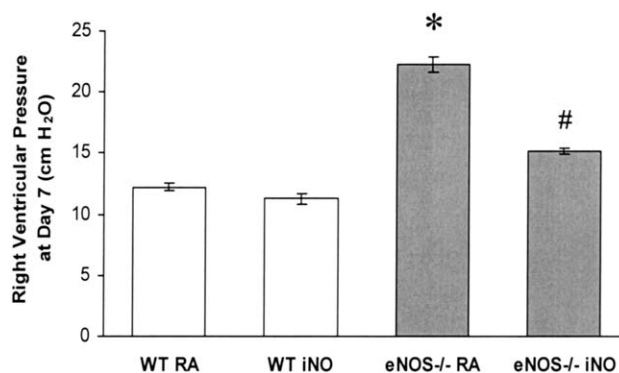


Fig 2. Right ventricular pressure (RVP) 7 days after left pneumonectomy in wild-type mice (WT [open bars]) and endothelial nitric oxide synthase knockout mice (eNOS^{-/-} [shaded bars]) recovered in either room air (RA) or 10 ppm inhaled NO (iNO). The RVP was measured through a closed chest, right ventricular cannulation technique. * $p = 0.0001$ versus all; # $p = 0.0001$ versus eNOS^{-/-} RA.

Right Ventricular Hypertrophy

Right ventricular hypertrophy was not observed, as the right-to-left ventricular weight ratios did not differ among groups at any timepoint (Fig 3).

Comment

We have shown that, after pneumonectomy, the RVP (and hence pulmonary artery pressure) transiently increases and then, under normal circumstances, returns to baseline level. In our study, RVP peaked at day 1 and returned to baseline by day 3 in WT mice. Our results correlate with data from Crouch and associates [12] who demonstrated an acute increase in pulmonary artery pressure from 14.0 to 18.8 cmH₂O in dogs after pneumonectomy. We did not find evidence of RVH in any mice after pneumonectomy. That may be due to the acute nature of this study (14 days after surgery), which may not have allowed for development of compensatory RVH despite the elevated RVP in eNOS^{-/-} mice. In fact, Cournand and associates [13] have shown that in patients

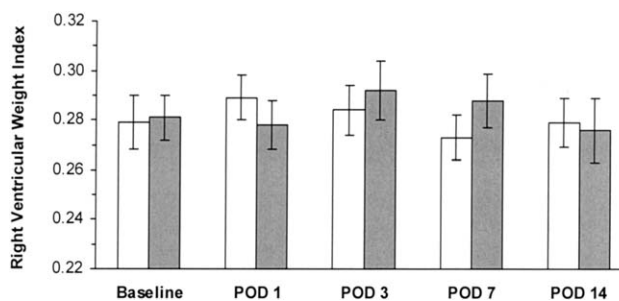


Fig 3. Right ventricle to left ventricle plus septum weight ratios after pneumonectomy in wild-type mice (WT [open bars]) and endothelial nitric oxide synthase knockout mice (eNOS^{-/-} [shaded bars]). There were no significant differences between groups at any timepoint, indicating absence of right ventricular hypertrophy. (Baseline = unoperated; POD = postoperative day.)

in whom pneumonectomy had been done 4 to 6 years previously, pulmonary hypertension did not occur despite years of increased pulmonary blood flow.

The pulmonary vasculature of the remaining lung adapts (vasodilates) and is able to tolerate a near doubling of the rate and volume of blood flow while maintaining normal pulmonary artery and right ventricular pressure. This phenomenon is most likely the result of many intrinsic cell signaling mechanisms initiated after pneumonectomy. These mechanisms, prominent of which is likely eNOS-regulated vasodilation, further influence vascular tone, airway compliance, and also compensatory lung growth. Endothelial cells lining the pulmonary microvasculature are subjected to increased intravascular pressure and shear stress associated with increased blood flow after pneumonectomy. Davies [14] has demonstrated that increased shear stress within the pulmonary microvasculature results in induction of various proteins and cell-signaling responses including the generation of reactive oxygen species and NO.

Studies from our laboratory have previously shown that pneumonectomy induces eNOS expression within the lung and that postpneumonectomy lung growth is significantly blunted in eNOS-/- mice [8]. The mechanisms that regulate and possibly link compensatory lung growth and postpneumonectomy pulmonary hypertension are intriguing but remain unknown. While this particular study does not specifically evaluate lung growth in the face of prolonged pulmonary hypertension, our findings pose an interesting link between our current conclusions and our previously published findings. In addition, although we did not measure residual lung wet-to-dry weight as a measurement of pulmonary edema, potential development of residual lung edema could also be a contributing factor to the increased pulmonary hypertension after pneumonectomy.

Three different NOS isoforms are involved in the production of NO. Two of these isoforms are constitutively expressed, primarily in endothelial and neuronal tissue (eNOS and nNOS, respectively) and are responsible for many of the beneficial properties of NO such as reduced vascular tone, prevention of neutrophil and platelet adhesion, and neuronal transmission. The third isoform, inducible NOS (iNOS), is induced at the transcriptional level by a number of proinflammatory stimuli including cytokines [15]. The principal isoform found in normal pulmonary vasculature is eNOS [16]. It is possible that there could be interactions between eNOS and iNOS that are yet to be described or explained. It is also possible that pneumonectomy results in acute pulmonary inflammation followed by induction of iNOS. If iNOS is induced after pneumonectomy, however, then this would likely blunt the level of RVP increase in both WT and eNOS-/- mice owing to vasodilatation properties of the induced NO. Further studies will be needed to determine if iNOS is induced after pneumonectomy and to explore possible contributions of iNOS to the postpneumonectomy vasoresponse.

The goal of the present study was to determine the role of an animal's intrinsic ability to generate NO after

pneumonectomy and correlate any deficiency in this system with postpneumonectomy pulmonary hypertension. We have demonstrated that eNOS-/- mice exhibit a transient postpneumonectomy hypertensive response not unlike that seen in wild type mice. The pulmonary hypertension observed in eNOS-/- mice, however, is sustained throughout the entire 14-day postoperative period and does not recover. This process can be significantly reversed with the use of low-dose iNO during the postpneumonectomy recovery period, further suggesting that NO is a key factor in compensatory vasodilatation after pneumonectomy. Also of interest is that recovery with iNO had no significant effect in WT mice RVP at day 7 after pneumonectomy compared with WT animals recovered in room air. In this study, we chose the 7-day timepoint to measure RVP after exposure to iNO simply because postpneumonectomy changes in RVP have reached a steady plateau in all mice by day 7, and it is on this day that the RVP returns to baseline values in the WT mice (see Fig 1).

The mechanisms by which NO, either inhaled or endothelial derived, participates in postpneumonectomy compensatory vasodilation are unknown at this time. Possibilities include vasodilation, decreased oxidative stress, decreased inflammation, improved gas exchange, or less likely, early angiogenesis. Further studies are ongoing to define more specific mechanisms in the postpneumonectomy model, in particular, the possible relationships between postpneumonectomy pulmonary hypertension and compensatory lung growth.

We conclude that a transient period of pulmonary hypertension follows pneumonectomy. Mice incapable of generating endothelial-derived NO maintain persistently high RVP for at least 2 weeks after pneumonectomy. Recovery with low-dose iNO, however, significantly blunts this response in eNOS-/- mice. These findings strongly suggest that eNOS is a key mediator in postpneumonectomy compensatory vasodilatation.

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